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14. ABSTRACT A comprehensive experimental model of controlled overpressure brain impact in rats is presented. Repeatable blast signatures of controlled duration, peak pressure and transmitted impulse enable to reproduce blast impact in laboratory animals in accurate fashion. Animal survival, brain pathomorphology and the expression of GFAP and CNPase after head-directed non-penetrating blast of 358 kPa magnitude at surface for approximately 10 msec. The high speed imaging demonstrated strong head acceleration/jolting accompanied by typical intracranial hematomas and brain swelling. The microscopic injury was revealed by prominent silver staining in the deep brain areas including nucleus subthalamicus zone suggesting both diffused and focal neurodegeneration. GFAP and CNPase, markers of astroglia and oligodendroglia, respectively, accumulated substantially in hippocampus 24 h after blast and persisted for 30 days post-blast. We propose that following blast overpressure brain impact, critical pathological signatures of blast brain injury may be triggered by complex cerebrovascular responses, including blood brain barrier disruption, glia activation and neuronal alterations.					
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Introduction

Identifying pathogenic pathways of primary blast brain injury (BBI) in reproducible experimental models is vital to the development of diagnostic algorithms for differentiating severe, moderate and mild 'organic' TBI (mTBI) from posttraumatic stress disorder (PTSD). Analysis of mechanisms and putative biomarkers of BBI is complicated by a deficiency of quality experimental studies. There is still a lack of pertinent reproducible models within the blast injury framework, including generators which precisely control parameters of the blast wave. This makes it difficult to predict the degree (mild vs. severe) of impact, which depends on characteristics of the blast wave affecting the body, for efficient development of diagnostics and mitigation. **The objectives** of this project has been to develop a comprehensive model with repeatable blast signatures of controlled duration, peak pressure and transmitted impulse enabling to reproduce blast impact in laboratory animals in accurate fashion. This will allow identifying pathogenic pathways of primary blast brain injury (BBI) and develop diagnostic algorithms for differentiating severe, moderate and mild 'organic' TBI (mTBI). The overall goal is to produce valuable diagnostics of blast brain injury.

BODY

Shock tube design, construction and setup

A compressed air-driven shock tube was used to expose rats to a supra-atmospheric wave of air pressure. The shock tube capable of generating a wide range of controlled blast waves without the use of explosives was designed, constructed and tested in collaboration with Florida Institute of Technology at Banyan Biomarkers, Inc. (**Fig.1A**).



Blast Generator Facilities at Banyan Biomarkers, Inc.



Fig. 1A. Overview of Blast Facilities at Banyan Biomarkers, Inc.

BLAST GENERATOR SET-UP



Fig.1B Blast Generator Set-Up.

The tube is separated in two sections: high-pressure (driver) and low-pressure (driven) separated by a metal diaphragm (**Fig. 1B**). The thickness, type of material, driver/driven ratio, and the initial driver pressure determines the peak and duration of the overpressure event. In the presented series of experiments 0.05 mm thick stainless steel diaphragms were used to generate high pressure shockwaves. The ratio of driver vs. driven section lengths was equal to 15. The driver section was initialized to a pressure of 5,170 kPa and the driven section was left to ambient conditions. The diaphragm rupture initiated by an internal cutter leads to the sudden exposure of a low pressure gas to a gas at significantly higher pressure resulting in the formation of a shock wave. The blast pressure data was acquired using PCB piezoelectric blast pressure transducers and LabView 8.2 software. A National Instruments 1.25 Msamples/sec data

acquisition card was used to acquire data from multiple channels. The rat head images during the blast event were captured at 1,000 frames/sec using a high speed video camera and Schlieren optics.

Blast wave characteristics

To study injury mechanisms and relevant biomarkers of blast brain injury, the characteristic parameters of the blast waves generated by the shock tube were first considered. The shock tube (Fig.1) was designed and built to imitate a freely expanding blast wave as generated by a typical explosion. Preliminary tests were conducted with no animal specimens to optimize the peak overpressure (OP) and exposure time to accurately reproduce blast events: driver pressure and volume, diaphragm material, and shock tube exit geometry. Following the diaphragm rupture, the driver gas sets up a series of pressure waves in the low pressure driven section that coalesced

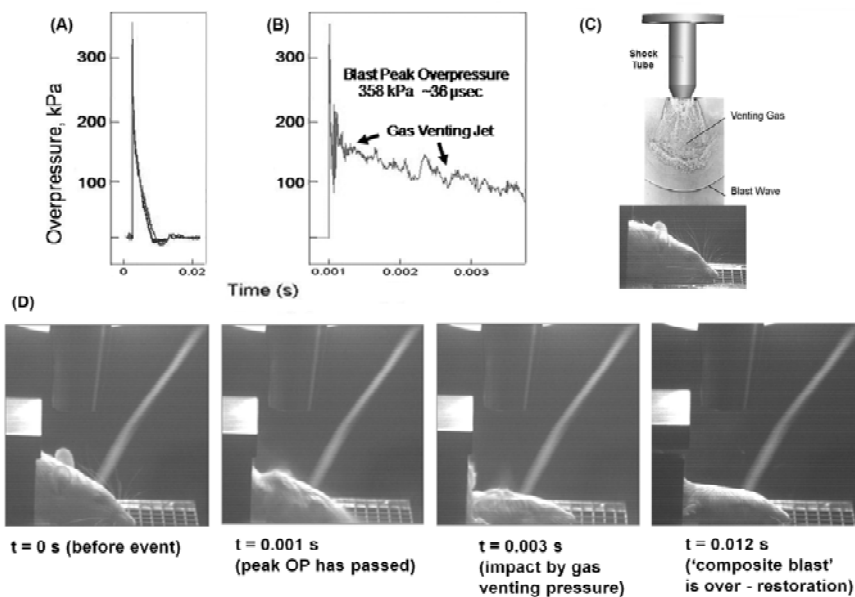


Figure 2. Experimental outline. (A): General shape of blast wave hitting an experimental animal showing a presence of negative phase; (B): Components of shock tube-generated blast wave; (C): Specimen positioning relative to shock tube, and (D): Rat head movement and deformation upon head-directed, body armored, blast wave exposure of 358 kPa for 10 msec.

to form the incident shockwave (Fig.2 A, B). The shockwave recorded by blast pressure transducers in the driven section and at the target showed three distinct events: (i) peak overpressure, (ii) gas venting jet and (iii) negative pressure phase-termed collectively “**composite blast**”. Peak overpressure, positive phase duration, and impulse appear to be the key parameters that

correlate to injury and likelihood of fatality in animals and humans, for various orientations of the specimen relative to the blast wave (1-6). A schematic of a shock tube nozzle and the rat

location relative to the shock tube axis, blast overpressure wave and gas venting cone is shown in Fig.2 C.

Several rat positions related to the nozzle of shock tube and head/body exposure protection has been studied (Fig. 3).

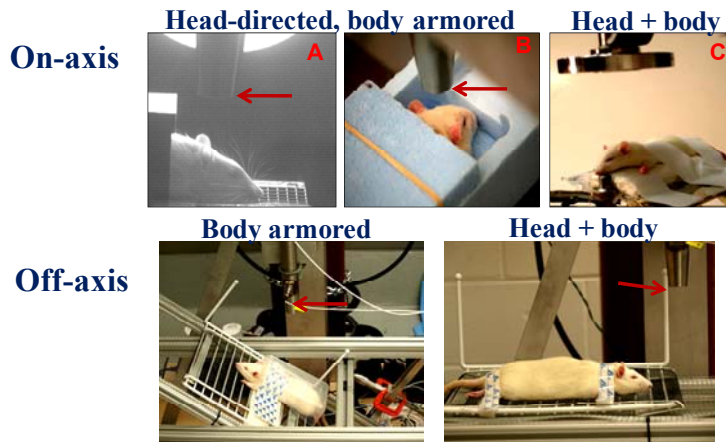


Fig. 3 Different rat positions toward shock tube exit nozzle: on-axis and off-axis

Animal exposure to a controlled blast wave.

All rats were anesthetized with isoflurane inhalations described previously in detail. After reaching a deep plane of anesthesia, they were placed into a holder exposing only their head (body-armored setup) at the distance 5 cm from the exit nozzle of the shock tube. Animals were then subjected to a single blast wave with a mean peak overpressure of 358 kPa, and positive pressure phase duration of approximately 10 msec (**Fig. 2**). Two control groups of animals, sham and naïve, underwent the same treatment (anesthesia, handling, recovery) except they were not exposed to blast. The rats in a sham group compared to naïve were exposed to the noise of a single blast at the 2 m distance from the shock tube while being anesthetized.

Also, several animals were exposed on axis at different blast magnitude/exposure time using head-directed, body armored impact (Fig.3B) vs. head directed open body impact (Fig.3C).

Effect of “composite blast” on rat survival upon total body vs. head-directed (body armored exposure.

We conducted experiments to compare rat survival upon blast exposure of open vs. armored body. First, the shock tube’s nozzle was directed to the rat’s head positioned at 5 cm from the opening, along the tube’s axis. After exposure of anesthetized rats with unprotected body to blast of 110 kPa (peak overpressure, OP) for 2 msec of composite blast wave, all rats remained alive during 24-48 hours post-blast (**Table 1**).

Table 1. Rat mortality after exposure of total body and head vs. body-armored to ‘composite blast’.

Peak Overpressure (kPa)	Total Blast Duration (msec)	Mortality
Total exposure (unprotected body)		
110 (n=3)	2	survived
170 (n=2)	4	lethal
358 (n=2)	1	lethal
Head-directed (body armored)		
172 (n=12)	4	all survived
358 (n=48)	10	all but one survived

Anesthetized rat were placed on solid platform in dorsal-up recumbence at different distances from the nozzle.

Rats were subjected to blast wave exposures of various magnitude and duration that included exposure to peak overpressure plus gas venting. Rats exhibited transitory symptoms of agitation within 15 to 30 min after

exposure during recovery from anesthesia (not shown). Further increase of OP magnitude to 190 kPa or 358 kPa for total blast duration of 4 and 1msec, respectively, resulted in the increase of rat mortality immediately after blast exposure (**Table 1**). By contrast, protecting the body and directing the blast to the head increased threshold of mortality and all rats remained alive after severe blast of 358 kPa peak OP and total duration of ~10 msec (Table 1).

Effect of “composite blast” on rats upon head-directed body protected exposure

Figure 2 D depicts rat head movement and deformation recorded by high speed video upon this severe head-directed blast wave exposure for 10 msec. Due to the complex nature of the blast event the brain injury is a result of a combined impact of the “composite” blast including all 3 major phases of a shockwave shown in **Fig. 2A and B**. Gas venting jet, albeit lower in magnitude, lasts the longest, represents the bulk of blast impulse and, possibly produces the most devastating impact. **Fig. 2D** demonstrates a strong downward head acceleration following the passage of peak overpressure which lasts ~36 μ sec. However, cranial deformation is more severe during the gas venting phase, lasting up to ~10 msec. Only when the positive pressure phase is over, the shape of the rat’s skull starts to restore. These points to a significant flaw in several previous studies described in the literature: animal specimens are usually placed along the axis of the shock wave generator. In such location, the venting gas jet creates a much larger impulse (mechanical energy transfer) in the specimen than the pressure shock wave itself. Such effect is

an experimental artifact since victims of explosive shock waves would not experience such venting gas jet.

Brain pathomorphology and histology

Head acceleration and deformation after severe blast exposure shown in Fig. 2 was accompanied by typical focal and massive intracranial hematomas and brain swelling (**Fig. 4B1,C1**).

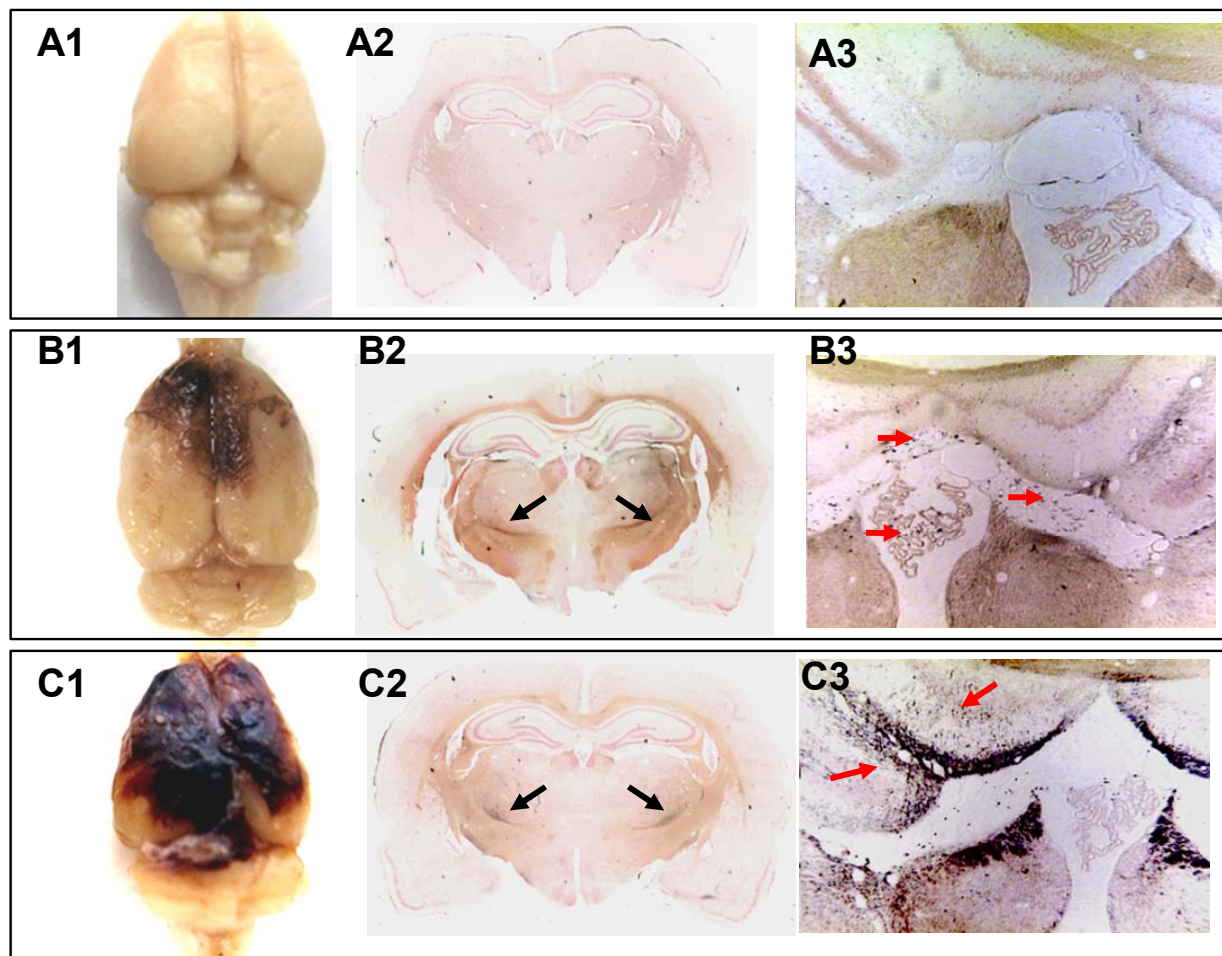
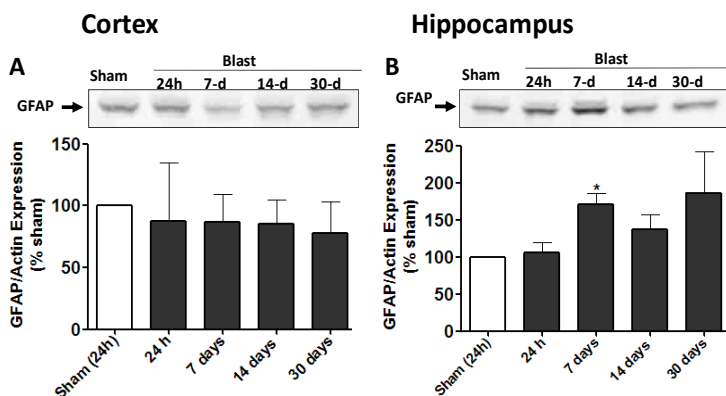


Figure 3. Brain pathomorphology after head-directed exposure to blast wave. Rats were subjected under anesthesia to head-directed blast impact (B and C), or noise (sham) (A). Forty eight hours after exposures (B) or at 5 day post-blast (C), brains were perfused in situ, removed and processed. A1-C1: Gross pathology: typical focal intracranial hematomas shown from at least 3 animals at each time point. Histopathology. A2-C2: coronal sections in caudal diencephalon exhibit diffuse and local silver accumulation. Arrows indicate strong silver staining in nucleus subthalamicus. Representative microphotographs of whole brains with high resolution scan (x1.5) are shown in the middle. A3-C3: 10x microphotographs of caudal diencephalon showing accumulation of silver-positive material in microvascular and perivascular beds (B3) and transition of positive silver staining into adjacent brain tissue (C3).

The hemorrhages and hematomas developed within hours after impact and appeared visibly through the undamaged skull at 24 to 48 hours after blast exposure (not shown). The size of hematomas varied significantly in different rats and formed a capsule at 5 day post-blast as shown in one the most damaged rat brain after in situ perfusion (**Fig. 4C1**). Coronal sections of brains fixed in situ by transcardial perfusion were stained for neurodegeneration using silver impregnation (10-12). Brain sections microscopy revealed a prominent silver becoming evident in the deep brain areas such as Caudal Diencephalon, including nucleus subthalamicus zone, 48 h post-blast (**Fig. 4B2**). The patterns of staining throughout the brain indicate both diffused and focal mild neurodegeneration, predominantly in the deep areas of rostral and caudal diencephalon (**Fig. 4B3,C3**) and mesencephalon (not shown).

GFAP and CNPase expression in cortex and hippocampus upon head-directed severe blast impact.

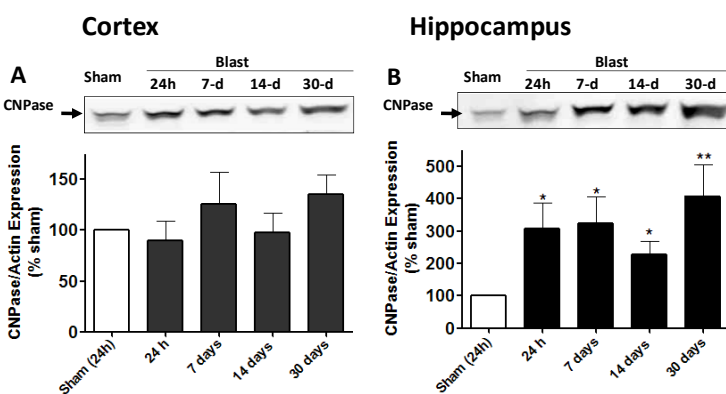
GFAP did not accumulate significantly in cortex within up to 30 days after blast (Fig. 5). There



was a slight increase in GFAP levels in hippocampus at 7 days post-blast with a tendency to further increase at day 30.

Fig. 5. Post-blast expression of GFAP in cortex and hippocampus.

By contrast, CNPase accumulated significantly in the cortex between 7 and 30 day post-blast



days post-blast (Fig. 6).

Fig. 6. Accumulation of CNPase in cortex and hippocampus following head-directed blast impact.

However, the most prominent, several-fold increase in CNPase expression was found in

hippocampus showing the maximal four-fold increase at 30 day after blast exposure (Fig. 6).

Drawbacks and solutions. As can be seen in Fig. 2, the effect of blast generated by the shock tube is confounded by ‘gas venting jet’. Normal explosions do produce blast winds that follow behind the incident shock (6). This effect is mimicked by shock tubes as the wave spherically expands. However, gas venting is an artifact inherent to shock tube operation, and not associated with the physics of actual blast events.

2. A novel solution is proposed to address this problem: placing the target at an off-axis

		Distance		Peak Over-pressure (psi/kPa)	Positive Phase Duration (μ s)	Impulse/Unit Area (kPa-s)
		D	cm			
	0°	2	5.08	52.6/361	NA	NA
	0°	4	10.16	20.3/140	NA	NA
level 1	30°	2.5	6.35	33.9/234	113.8	10.6
	30°	4	10.16	17.7/122	138.0	8.37
	45°	2	5.08	35.2/243	53.1	6.46
level 2	45°	4	10.16	13.8/95	85.3	4.04
	60°	2	5.08	24.5/169	32.9	2.76
level 3	60°	4	10.16	10.6/73	60.3	2.20

angle avoids venting altogether.

Measurements of blast traces were made along the device axis ($\theta=0^\circ$) and off-axis ($\theta=30, 45, 60^\circ$) at several distances from the exit: the results are summarized in Table 2.

The changing local speed of sound behind the wave causes the duration to increase with distance. For example, the 45° data

shows duration increases from 53.1 to 85.3 μ s as the distance increases from 2D to 4D. By varying pressure settings, driven and driver lengths, and specimen location, independent control of blast overpressure, duration, and impulses will be achieved.

3. During next year of this project, we will subject rats to an off-axis blast of different magnitude and duration at various distances from blast wave generator to the rat. As seen in the Table 3, we will begin from 3 different settings to determine the severity of real blast exposure: level 1 (presumably moderate to severe exposure), level 2 (presumably mild to moderate exposure) and level 3 (mild exposure). As can be seen, the strength of blast is determined by a combination of peak overpressure magnitude and duration that determines impulse. Thus, we will establish a Blast Impact Index (BII) that will reflect a threshold of blast strength that produces mild TBI upon single exposure when blast hits whole body vs. local, head-directed blast wave exposure. Then, we will determine the corresponding BII necessary to produce mTBI upon repeated exposures. Brain tissue, CSF and blood will be taken for analysis of biochemical markers (please see below) and the levels of brain tissue injury will be assessed using silver staining as we described previously (10-12) with the assignment of appropriate score for damage ranking from 0 (not detectible) to 5 (severe). We will also employ histopathology, silver staining,

Fluoro-Jade and vasospasm staining to assess the presence and the level of brain injury. This effect can be virtually eliminated by placing the specimens off-axis from the venting jet, in a way that the main effect acting on the specimen is the over pressure event (shock wave).

Key Research Accomplishments:

- A comprehensive experimental model of blast exposure has been developed in collaboration with the Department of Aerospace and Mechanical Engineering, Florida Institute of Technology
- By varying pressure settings, driven and driver lengths, and animal location, independent control of blast overpressure, duration, and impulses has been achieved. We defined Blast Impact Index (BII) as a combined function of blast wave magnitude at the body surface (peak overpressure), duration and impulse power.
- Total body blast exposure had a much greater impact on mortality compared to head-directed exposure of a similar magnitude: body protection dramatically increased rat survival.
- The high speed imaging reflected strong head acceleration upon on-axis head-directed blast, and brain pathomorphology showed typical massive and focal intracranial hematomas and brain swelling.
- This severe damage was accompanied by strong positive silver staining in the several deep brain areas including Diencephalon (e.g. Nucleus Subthalamicus zone) indicating both diffused and focal neurodegeneration.
- GFAP, a marker of astroglia, increased modestly in hippocampus, but not in cortex, at 7 day after blast exposure and appeared to persist up to 30 days post-blast.
- CNPase, a marker of oligodendroglia, strongly accumulated in hippocampus 24 hours after blast and remained highly elevated up to 30 days post-blast.

Reportable Outcomes

1. The review article entitled “**Biomarkers of Blast-Induced Neurotrauma: Profiling Molecular and Cellular Mechanisms of Blast Brain Injury**” by Svetlov SI, Lerner SF, Kirk DR, et al. has been published *J Neurotrauma* 2009, 26:1-9
2. The paper entitled “**Morphologic and biochemical characterization of brain injury in a model of controlled blast overpressure impact**” by Stanislav I. Svetlov, Victor Prima, Daniel R. Kirk, Joseph Atkinson, Hector Gutierrez, Kenneth C. Curley, Ronald L. Hayes, Kevin K.W. Wang has been submitted to *J. of Trauma* (#JT-S-09-00665).
3. The poster entitled “**A novel model of blast overpressure brain injury: a comprehensive platform for profiling molecular and cellular mechanisms and systems biology studies**” was presented at 26th Annual National Neurotrauma Symposium, July 27-30, 2008, Orlando FL.
4. Abstract submitted for the Military Health Research Forum 2009 in Kansas-City, MO has been accepted for oral presentation: **Blast-Induced Neurotrauma: Comprehensive Experimental Models for Profiling Mechanisms and Developing Biomarkers of Blast Brain Injury**
5. Abstract entitled “**Morphological and Biochemical Signatures of Brain Injury Following Head-Directed Controlled Blast Overpressure Impact**” has been accepted for poster presentation at 27th Annual national Neurotrauma Symposium, September 7-11, 2009, Santa Barbara CA.

Conclusion

In first year of the project, we developed and employed a model of 'composite' blast exposure with controlled parameters of blast wave impact and brain injury in rats for studies of mechanisms and biomarkers of BBI. We demonstrate that brain damage induced by severe head-directed blast waves is accompanied by time-dependent intracranial hemorrhages and neurodegeneration in deep areas of brain. This was accompanied by the accumulation of GFAP and CNPase predominantly in hippocampus. The data suggests that mechanisms underlying blast brain injuries, may be distinct from those imposed by mechanical impact, and may be triggered by systemic, cerebrovascular and neuro-glia responses as consecutive but overlapping events.

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